

# Comet Assay for DNA Damage

JW Jianmin Wan

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 An abbreviated version of this protocol was published in Plant Physiology in Sep 2019

Earlier Degraded Tapetum1 (EDT1) Encodes an ATP-Citrate Lyase Required for Tapetum Programmed Cell Death1,[OPEN]

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## Detailed protocol

### Comet Assay for DNA Damage

The comet assay was performed using the CometAssay Kit (Trevigen) with minor modifications.

(1) Cracking. Place a petri dish containing 1 mL of ice-cold solution (1x phosphate-buffered saline and 20 mM EDTA). Chop 0.2 g of anther sample in the petri dish with a scalpel. A 100-mm nylon mesh filter (Millipore) was used to filter the nuclei mixed.

(2) Sample loading. 50 µL of the nuclei mixed into 300 µL of 1% (w/v) low-melting agarose (LMA agarose, pre-incubated at 37°C) and pipetted onto slides.

After coagulation, immerse the slides in 4°C lysis solution (kit provided) for 1 h. Pay attention to handle all process gently.

Note: the LMA agarose should be melted at 90–100°C water bath for 5 minutes and placed in a 37°C water bath for at least 20 minutes to cool before use.

(3) Unspin. Drain excess buffer from slides and immerse in freshly prepared Alkaline Unwinding Solution (0.3 N NaOH, 5 mM EDTA) for 40 minutes at 4°C, in the dark. Then wash the slides with 1x TBE for 5min and repeat 2 times.

(4) Electrophoresis. Take out the slides and slides were electrophoresed at 25 V in 1x TBE for 30 min and then immersed in 70% (v/v) ethanol for 5 min.

(5) Dyeing and take pictures. After drying at room temperature, the slides were stained with SYBR green (1:10,000 dilution; Bio-Rad) and then examined with a Zeiss ImagerA2 microscope. The CometScore software (<http://www.autocomet.com>) was used to evaluate the percentage of DNA in each comet tail. Use CometScore software (<http://www.autocomet.com>) to evaluate the percentage of DNA (T DNA%) in each comet tail. The degree of DNA damage of different samples was calculated according to the method described by Wang and Liu (2006).

**How to cite:** (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Wan, J. (2021). Comet Assay for DNA Damage. Bio-protocol Preprint. [bio-protocol.org/prep1321](https://bio-protocol.org/prep1321).
2. Bai, W., Wang, P., Hong, J., Kong, W., Xiao, Y., Yu, X., Zheng, H., You, S., Lu, J., Lei, D., Wang, C., Wang, Q., Liu, S., Liu, X., Tian, Y., Chen, L., Jiang, L., Zhao, Z., Wu, C. and Wan, J. (2019). Earlier Degraded Tapetum1 (EDT1) Encodes an ATP-Citrate Lyase Required for Tapetum Programmed Cell Death1,[OPEN]. Plant Physiology 181(3). DOI: [10.1104/pp.19.00202](https://doi.org/10.1104/pp.19.00202)

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